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The Effects of Thyroxine and Propylthiouracil Treatment on Changes in Body Form Associated with a Possible Developmental Thyroxine Surge During Post-hatching Development of the Tilapia, *Oreochromis mossambicus*

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ABSTRACT—In tilapia, *Oreochromis mossambicus*, a surge in thyroxine (T_4) begins near the time of complete yolk absorption (10 days after hatching). We were interested in examining the effects of physiological changes in T_4 on growth and body morphology of this species. To this end, starting 3 days after hatching, larvae were immersed in propylthiouracil (PTU, 50 ppm) or in physiological doses of T_4 (2.0, 5.0 and 20 ng/ml of aquarium water). Whole-body thyroid hormone levels and morphometric parameters were determined throughout the first 38 days after hatching. Thyroxine content was significantly increased in larvae exposed to 5.0 or 20 ng/ml T_4 compared with controls, with the highest levels observed during the time of the natural T_4 peak (day 10–28 posthatch). By contrast, T_4 content was significantly decreased in PTU-treated larvae at this time. These results indicate a role of the thyroid for the T_4 surge. The T_3 content of larvae exposed to 20 ng/ml T_4 was significantly increased compared with controls. Exposure to T_4 significantly altered body proportions. Both the anal depth to standard length ratio and body area of larvae were significantly increased by 20 ng/ml T_4 treatment and decreased by PTU treatment. This suggests a role of the T_4 surge in the metamorphosis from a slender, elongated body form to a deep-bodied juvenile form. There were no observed effects of hormone treatment on other morphological features that were examined including body weight, standard length, and the rate of yolk-sac absorption.

INTRODUCTION

Thyroid hormones are well established as controlling factors in vertebrate morphogenesis and metamorphosis. A common feature preceding many of these metamorphic events is a surge in thyroxine (T_4). In teleost fishes, T_4 surges have been correlated with salmon smoltification [5] and, more recently, flounder metamorphosis [4, 14, 22, 24].

Thyroid hormone surges occurring near the time of complete yolk-sac absorption have recently been identified in several teleost species [2, 8, 20]. The nature and physiological significance of these increases in thyroid hormone have been the focus of much speculation. Kobuke *et al.* [8] were the first to identify such an increase in whole-body T_4 concentration in coho salmon. Shortly thereafter, similar increases in whole-body T_4 concentrations were reported for striped bass [2], chum and chinook salmon [6, 20], and in whole-body triiodothyronine (T_3) concentrations for striped bass [2], coho and chum salmon [6, 21]. The cause of these increases in thyroid hormone have been attributed to the onset of thyroxinogenesis and to stores of thyroid hormone in the yolk [2, 6]. Whatever their source, these increases in thyroid hormone have been correlated with rises in the growth rate and the feeding rate [8, 20]; however, no morphological changes have been described.

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Recently, a T_4 peak starting near the completion of yolk absorption (10 days after hatching) was observed in the tilapia (*Oreochromis mossambicus*) [16, 18]. Reddy *et al.* [18] correlated this T_4 surge with an increase in larval growth rate and the onset of feeding and free-swimming behavior. Furthermore, Reddy *et al.* [17] demonstrated that the growth rate and the onset of swimming behavior can be accelerated by exogenous thyroid hormone treatment at this time. In tilapia larvae, thyroid hormone treatment has also been shown to affect fin differentiation as well as growth, body pigmentation and silvering, and epidermal thickening [9, 10, 15, 17]. In addition, treatment of milkfish (*Chanos chanos*) and red sea bream (*Pagrus major*) postlarvae with T_4 has been shown to accelerate the transition to the juvenile form [7, 11].

The preceding studies demonstrate that thyroid hormone surges occur near the time of complete yolk-sac absorption in several teleost species, including tilapia, and that the larvae are responsive to exogenous thyroid hormone treatment at this time. It has yet to be demonstrated whether this T_4 surge is involved in the metamorphosis of this species, which we define as a transition in body form from a slender, elongated larvae to a shorter, deep-bodied juvenile. To examine this possibility, we manipulated T_4 levels at the time they normally rise when yolk absorption is nearly complete. This was accomplished through exogenous treatment with T_4 or propylthiouracil (PTU), an antithyroid drug. Changes in body morphology indicative of the larval to juvenile transition were examined.

MATERIALS AND METHODS

Thyroid hormone profiles during post-hatching development

Four clutches of fertilized eggs (expected to hatch within 24 hr), ranging in size from 400–500 eggs per clutch, were collected from mouthbrooding females maintained in a freshwater tank at the Hawaii Institute of Marine Biology of the University of Hawaii, Oahu. Each clutch was placed in a circular, 10 liter plastic tank maintained at $27.0 \pm 0.5^{\circ}C$ and exposed to a 14:10 light-dark cycle. Larvae were fed ground Tetra Min Flake food to satiety twice a day starting 8 days after hatching, which was 2 days prior to complete yolk absorption. Water in the tank was renewed daily with water from a holding tank maintained at the same temperature. Five to twenty larvae per clutch were sampled at intervals during 0–38 days after hatching. Larvae were weighed before storing at $-80^{\circ}C$ for subsequent thyroid hormone analyses.

Effects of T_4 and PTU treatment on thyroid hormone concentration, growth, and body morphology in larvae

Six to eight clutches of fertilized tilapia eggs were collected and incubated together in a hatching tank. Clutches of eggs that hatched within 24 hr of one another were pooled and randomly distributed into 5 groups of 300 larvae each. Each group was placed into a 10 liter tank containing water with either no added T_4 or PTU (control), T_4 at concentrations of 2.0, 5.0, or 20 ng/ml of aquaria water, or PTU at a dose of 50 ppm. The range of T_4 doses chosen in this study were based on the T_4 content of larvae in the previous experiment, with the highest T_4 dose being approximately 8 times higher than peak endogenous T_4 levels (assuming ng/ml = ng/g). A T_4 stock solution was prepared by adding 1.0 mg of thyroxine sodium salt (Sigma, St. Louis, MO) to 100 ml of distilled water. One drop of 10 N NaOH was added to the solution (to facilitate hormone solubility) and stirred for 10 min. Volumes of 0.6, 1.5 and 6.0 ml of this stock solution were diluted in 3 liters of tap water to give the final T_4 concentrations of 2.0, 5.0 and 20 ng/ml in the aquarium water, respectively. The PTU medium was prepared by dissolving 150 mg of PTU (Sigma) in 3 liters of tap water. Thyroxine and PTU were renewed daily following a complete water change to maintain the hormone levels in the tank water. To ascertain T_4 levels in tanks, water samples were taken from untreated and T_4-treated tanks immediately following addition of T_4 to the tank water and again prior to water change 24 hr later (Table 1). The calculated, not actual, T_4 concentrations are shown in the figure legends. Hormone treatment was initiated 3 days after hatching and continued to the end of the

study. Larvae were maintained under environmental conditions and feeding schedules similar to those described above. Five to twenty larvae per treatment were sampled at intervals during 2–38 days after hatching. Larvae were measured and weighed before storing at −80°C for thyroid hormone analyses. The study was repeated for a total of four trials.

Thyroid hormone extraction

Thyroid hormones contained in frozen larvae were extracted following the methods described by Tagawa and Hirano [20] and Weber *et al.* [25], except that 95% ethanol was substituted for methanol in the extraction solution. Extraction efficiency based on recovery of radiolabeled T_3 tracer was 86.1±0.50% (mean±SE).

T_4 and T_3 radioimmunoassay

Thyroid hormones in larval tilapia were quantified using methods described by Brown and Eales [3] and Weber *et al.* [25], but with the following modifications:

1. Disposable polyethylene filter columns (QS-24 G-25 sephadex columns, Isolab Inc., Akron, OH) were used in place of 5 ml barrel syringes.
2. Phosphate buffer (0.1 M sodium phosphate dibasic heptahydrate, 0.1 mM disodium ethylenediamine tetraacetate in deionized distilled water, pH adjusted to 7.6 with 10 N NaOH) was used in both the T_4 and T_3 assay.
3. The volume of buffer used for the first elution containing the radioiodide contaminants and then for the elution of the antibody-bound fraction was 2 ml for both assays.
4. The columns were regenerated by elution of 4 ml of distilled deionized water, followed by 2 ml of thyroid hormone-stripped human serum (Endocrine Sciences, Tarzana, CA) diluted 1:20 with phosphate buffer, 8 ml of deionized distilled water and 8 ml of 0.1 N NaOH.

A volume of 50 μl of sample and standard was used for both assays. Samples and standards were assayed in triplicate and 5 columns were designated for sample pools to determine intraassay and interassay variability. The intraassay coefficient of variation was 7.8% and 7.1% for T_4 and T_3 (n=5), while the interassay coefficient of variation was 9.6% and 7.8% for T_4 and T_3 (n=5), respectively.

The competitive binding curves for T_4 and T_3 in the larval extract pool exhibited good parallelism with the T_4 and T_3 standard curves, respectively (data not shown). The possible effects of sample volume on thyroid hormone measurement were also examined. Variations in the sample volume from 25–100 μl did not alter measurement of T_4 and T_3 in the larval extract pool within the ranges tested.

Individual extraction recovery values were used to calculate hormone concentrations. Thyroid hormone content per larva was determined by multiplying the thyroid hormone concentration by the mass of the individual larva.

Morphometric measurement of tilapia larvae

Figure 1 shows the types of morphological measurements made on larval tilapia using a Zidas digitizer (Carl Zeiss, Inc., Thornwood, NY) and Wild Herrburg M20 dissecting scope outfitted with a camera lucida attachment. In addition, several ratios of different body measurements that in-

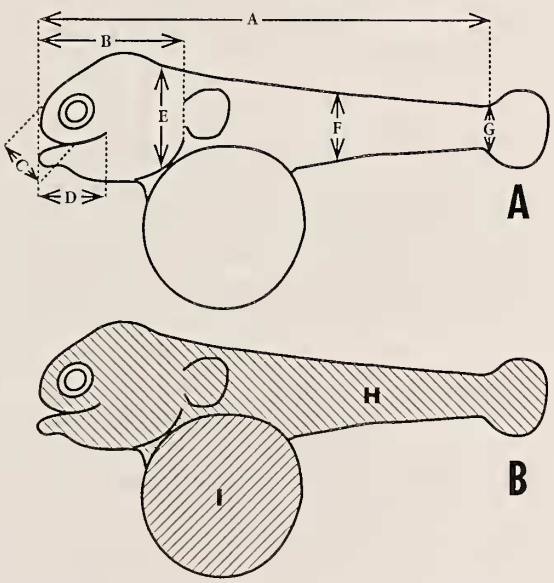

FIG. 1. Schematic diagram of different morphometric measurements made on *Oreochromis mossambicus* larvae. A. A, standard length; B, snout-to-operculum length; C, upper jaw length; D, lower jaw length; E, operculum depth; F, anal depth; G, caudal peduncle depth. B. H, body area; I, yolk-sac area.

cluded the operculum depth to standard length, the anal depth to standard length and the caudal peduncle depth to standard length ratios were calculated to obtain information on changes in body proportion. Six to nine larvae per treatment (n=36 total) were measured at each sampling at designated intervals during 2–38 days after hatching, prior to being weighed and frozen for thyroid hormone measurement.

Statistical analysis

Differences in thyroid hormone levels within treatments were analyzed using a one-way analysis of variance (ANOVA) and the least significant difference test (LSD) for a *priori* pair-wise comparisons [19]. Differences in thyroid hormone levels, body weight and morphometric measurements between treatments were evaluated using a two-way ANOVA and the LSD test for a *priori* pair-wise comparisons [19]. All results were considered significant at $P<0.05$.

RESULTS

T_4 concentration in tank water

Table 1 shows the T_4 levels in aquaria water from the control and T_4-treated tanks. There was no T_4 detected in the water of the control tank. Thyroxine levels in water from 2.0, 5.0 and 20 ng/ml T_4-treated tanks contained approximately 1.2, 4.0 and 14.8 ng/ml T_4, respectively, immediately following addition of hormone to the water. These levels fell to approximately 0.5, 1.2 and 2.6 ng/ml T_4, respectively, prior to the next water change 24 hr later. This overnight decline in the T_4 levels may result from a variety of factors that include metabolism by fish larvae, binding of T_4 to tank walls and degradation by bacteria.

Table 1. T_4 concentration of aquarium water from control, 2.0 ng/ml, 5.0 ng/ml and 20 ng/ml T_4-treated tanks sampled immediately after addition of hormone to the tank water and 24 hr later (mean±SE, n=10)

Treatment	T_4 (ng/ml)	
	Immediately after hormone addition	24 hr later
Control	0	0
2.0 ng/ml T_4	1.2±0.12	0.5±0.07
5.0 ng/ml T_4	4.0±0.23	1.2±0.22
20 ng/ml T_4	14.8±0.77	2.6±0.54

Thyroid hormone profiles during post-hatching development

Figure 2A illustrates the changes in whole-body T_4 and T_3 concentrations of larval tilapia during 0-38 days after hatching expressed as hormone per g body weight. Whole-body T_4 concentration of larvae at the time of hatching was 2.60±0.10 ng/g (mean±SE). The T_4 concentration dropped significantly ($P<0.01$) to 0.86±0.04 ng/g by 9 days post-hatching and then increased sharply to a peak level of 3.70±0.50 ng/g by 18 days post-hatching. This level was not significantly different from that of newly-hatched larvae, but it was significantly higher ($P<0.01$) than that of 9-day-old larvae. Thereafter, the T_4 concentration decreased to less than 1.0 ng/g by 28- and 38 days post-hatching.

The whole-body T_3 concentration of larvae at the time of hatching was 10.17±0.35 ng/g, approximately 4 times higher than that of the T_4 concentration. Thereafter, the T_3 concentration dropped significantly ($P<0.01$) to 2.14±0.34 ng/g by 9 days post-hatching and reached a level of 0.77±0.20 ng/g by 28 days post-hatching.

Figure 2B shows the thyroid hormone levels in larval tilapia during 0–38 days after hatching expressed as total hormone content (ng) per larva. The T_4 content in larvae at the time of hatching was 0.014±0.001 ng/larva (mean±SE). Nine days after hatching, the T_4 content in larvae started to increase and reached peak levels of 0.087±0.015. The T_4 contents were 0.087±0.015, 0.073±0.015 and 0.079±0.023 ng/larva by 18-, 21- and 24 days post-hatching, respectively, which were significantly higher ($P<0.01$) compared with newly-hatched larvae. Thereafter, there was a significant drop ($P<0.05$) in the T_4 content by 28 days post-hatching to 0.035±0.015 ng/larva. The T_4 content significantly increased ($P<0.05$) to 0.085±0.024 ng/larva by 38 days post-hatching.

The T_3 content decreased significantly ($P<0.01$) from 0.050±0.002 ng/larva at the time of hatching to 0.023±0.003 ng/larva by 9 days post-hatching.

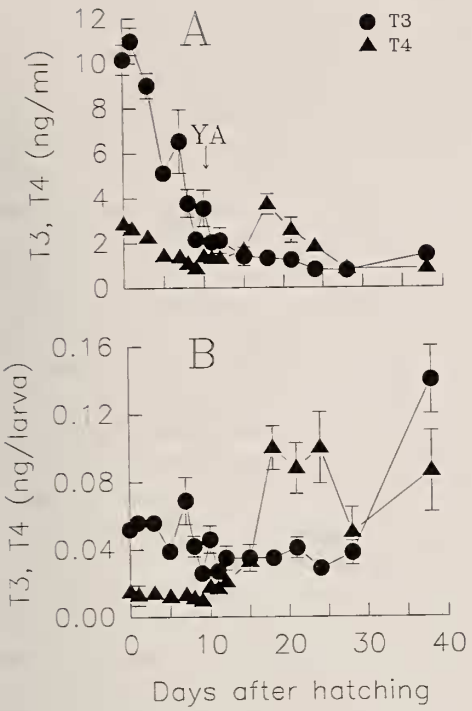

FIG. 2. A. Changes in T_4 (solid triangles) and T_3 (solid circles) tissue concentrations in larval tilapia during 0–38 days after hatching (mean±SE, n=4). Complete yolk absorption (YA) occurred 10 days after hatching. B. Changes in T_4 (solid triangles) and T_3 (solid circles) content (represented as ng/larva) in tilapia larvae during 0–38 days after hatching (mean ±SE, n=4).

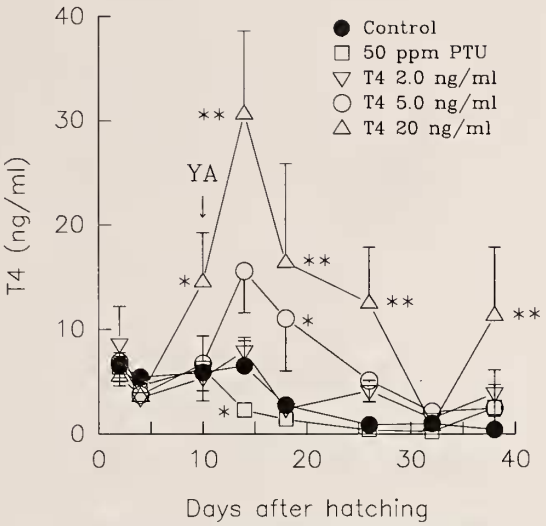

FIG. 3. Effects of T_4 and PTU treatment on the T_4 tissue concentration of tilapia larvae during 2–38 days after hatching (mean±SE, n=4). Complete yolk absorption (YA) occurred 10 days after hatching. Larvae were reared in water containing no T_4 or PTU (solid circles, dashed line), T_4 at doses of 2.0 ng/ml (open triangles), 5.0 ng/ml (open squares) and 20 ng/ml (open circles), and PTU at a dose of 50 ppm (open diamonds) starting 3 days after hatching. Asterisks denote significant differences (*: $P<0.05$, **: $P<0.01$).

The T_3 content increased sharply to 0.122±0.015 ng/larva by 38 days post-hatching, which was significantly higher ($P<0.01$) compared with 28-day-old larvae.

Effects of T_4 and PTU treatment on thyroid hormone concentration in larvae

The effects of exogenous T_4 and PTU treatment on the T_4 tissue concentration in larval tilapia from 2–38 days after hatching are shown in Figure 3. The T_4 concentration in larvae treated with 20 ng/ml T_4 was significantly elevated ($P<0.05$) compared with controls by 10-, 14-, 18-, 26- and 38 days post-hatching. The T_4 concentration of the 5.0 ng/ml T_4-treated larvae was significantly higher ($P<0.05$) compared with controls by 18 days after hatching. Fourteen days after hatching, the T_4 concentration of larvae treated with 50 ppm PTU was significantly lower ($P<0.05$) compared with controls. The T_4 concentration of 2.0 ng/ml T_4-treated larvae was not significantly different compared with controls.

Figure 4 shows the effects of exogenous T_4 and PTU treatment on the T_3 tissue concentration of larval tilapia from 2–38 days after hatching. The T_3 concentration of the 20 ng/ml T_4-treated larvae taken overall (2–38 days post-hatch) was significantly greater ($P<0.05$) than that of controls, but the difference between treated and control fish was not significant at any specific time period. Larvae reared in 50 ppm PTU, or in 2.0 or 5.0 ng/ml T_4, were not significantly different in the T_3 concentration compared with controls.

Effects of T_4 and PTU treatment on larval growth and development

Young larvae (<32 days post-hatch) treated

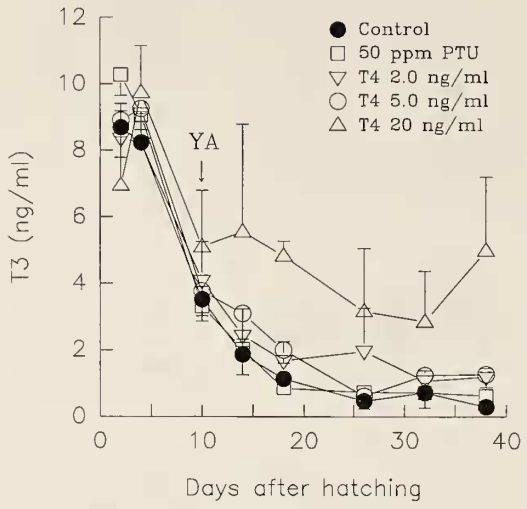

Fig. 4. Effects of T_4 and PTU treatment on the T_3 tissue concentration of tilapia larvae during 2–38 days after hatching (mean±SE, n=4). Complete yolk absorption (YA) occurred 10 days after hatching. Larvae were reared in water containing no T_4 (solid circles, dashed line), T_4 at doses of 2.0 ng/ml (open triangles), 5.0 ng/ml (open squares) and 20 ng/ml (open circles), and PTU at a dose of 50 ppm (open diamonds) starting 3 days after hatching.

with T_4 and PTU were not significantly different compared with controls in body weight and measured morphological characteristics (data not shown). Table 2 shows the effects of T_4 and PTU treatment on external body morphology of 32- and 38-day-old larvae. Larvae treated with 20 ng/ml T_4 were significantly greater ($P<0.05$) in body area compared with controls by 32- and 38 days after hatching, whereas the 2.0 and 5.0 ng/ml T_4-treated larvae were significantly smaller ($P<0.05$) in body area compared with controls over the same time periods. The PTU-treated larvae were significantly smaller ($P<0.05$) in body area compared with controls by 38 days after hatching. The 20 ng/ml T_4-treated larvae were also significantly greater ($P<0.05$) in the anal depth to standard length ratio compared with controls by 38 days after hatching, whereas larvae treated with 5.0 ng/ml T_4 and PTU were significantly smaller ($P<0.05$) in the anal depth to standard length ratio compared with controls by 32- and 38 days after hatching. The 2.0 ng/ml T_4-treated larvae were significantly smaller ($P<0.05$) compared with controls at 32 days after hatching. Analysis of remaining body measurements made on larvae revealed no significant differences between treated (T_4 and PTU) and untreated larval tilapia.

Table 2. Effects of T_4 and PTU treatment on external body morphology of *Oreochromis mossambicus* larvae at 32 and 38 days after hatching. Values are expressed as means±SE

Treatment	n	Body weight (g/larva)	Standard length (mm)	Anal depth (mm)	Anal depth/ standard length (mm)	Body area (mm^2)
			Day 32 after hatching			
Control	35	0.037±0.004	10.83±0.04	2.78±0.09	0.313±0.059	39.0±2.41
50 ppm PTU	36	0.039±0.004	10.86±0.23	2.71±0.09	0.247±0.003*	37.13±2.09
2 ng/ml T_4	36	0.041±0.006	10.42±0.26	2.59±0.10	0.246±0.004*	34.22±2.11*
5 ng/ml T_4	35	0.037±0.004	10.30±0.22	2.52±0.08	0.243±0.003*	34.25±1.74*
20 ng/ml T_4	26	0.053±0.004	10.90±0.60	3.07±0.08	0.444±0.085	45.82±1.77*
			Day 38 after hatching			
Control	36	0.069±0.008	12.76±0.30	3.42±0.12	0.266±0.003	54.72±3.12
50 ppm PTU	36	0.062±0.007	12.31±0.32	3.17±0.11	0.257±0.003*	49.19±2.88*
2 ng/ml T_4	36	0.065±0.010	12.36±0.67	3.12±0.12	0.271±0.018	48.57±2.85*
5 ng/ml T_4	36	0.070±0.010	12.02±0.32	3.12±0.11	0.258±0.003*	49.52±2.86*
20 ng/ml T_4	36	0.081±0.011	13.38±0.66	3.78±0.17	0.362±0.076*	62.56±5.84*

* $P<0.05$ compared to control

DISCUSSION

The thyroid hormone patterns observed in this study are consistent with those of Reddy [18] and Weber *et al.* [25] who detected the presence of substantial amounts of thyroid hormones in eggs and larvae during early development of tilapia (*O. mossambicus*). However, comparison of thyroid hormone values in this study with those reported by Reddy *et al.* [18] suggests that there are marked intraspecies differences in the relative levels of thyroid hormones present in tilapia eggs and larvae, despite the reproducibility in the pattern of T_4 and T_3 profiles during post-hatching development. In the present study, the T_3 concentration in larvae at the time of hatching was greater than the T_4 concentration, whereas Reddy [18] found that T_4 content was higher than T_3. This discrepancy in larval thyroid hormone levels may be related to differences in the environmental history of the breeding females. Mature females used in our study were captured in seawater and acclimated to fresh water for a minimum of 2 months. In contrast, Reddy [18] used females reared in fresh water (Lam, personal communication). Support for this explanation comes from a study by Tagawa *et al.* [23] who reported that eggs and larvae of marine teleosts had greater T_3 concentrations than T_4, whereas most fresh water fish had greater concentrations of T_4 than T_3. The biological significance of different T_3/T_4 ratios between freshwater- and seawater-adapted teleosts of the same species is not known and requires further investigation. Intraspecies variations in the levels of thyroid hormones have also been reported for several salmonid species [12, 13].

A steady decline in larval T_4 and T_3 concentrations from hatching until 9 days after hatching were observed in this study as well as by Reddy *et al.* [18]. Similar decreases in the T_4 content were reported in coho salmon, *Oncorhynchus kisutch* [8], chum salmon, *O. keta* [6, 20] and striped bass, *Morone sexatilis* [2]. It has been suggested that this decline in thyroid hormone concentration during the period of yolk-sac resorption results from the hormone utilization by developing larvae [1]. However, the T_3 content, but not the T_4 content, in the tilapia decreased after hatching, suggesting that T_3 is mainly utilized during this period of larval development. Further support comes from Reddy *et al.* [18] who found that T_4 5′-deiodinase (5′-D) activity is nondetectable until 5 days after hatching. Thus, T_3 stored in eggs may act as an important source of hormone during early post-hatch development, while T_4 may be utilized at a latter period.

The marked increase in the T_4 level starting around the period of complete yolk-sac absorption (10 days after hatching) in *O. mossambicus* suggests the beginning of endogenous secretion of T_4 from the larval thyroid. The drop in T_4 concentration in PTU-treated larvae suggests utilization of T_4 at this time, eliminating decreased utilization of T_4 as a cause of the T_4 surge. As mentioned earlier, similar increases in the T_4 concentration occurring at or near complete yolk absorption have been reported in coho and chum salmon which coincide with an increase in the growth rate of these teleosts [8, 20]. This correlation was also found in tilapia [18].

The significant decrease in T_4 following the marked increase may reflect an excess of conversion to T_3 over secretion of T_4 from the larval thyroid due to a noticeable increase in 5′-D activity [18]. In addition, we have found that the thyroid hormone level remained depressed following the T_4 surge when expressed in units of ng/g (Fig. 2a), but it increased significantly when expressed as a function of ng/larva (Fig. 2b). This suggests that thyroid hormone synthesis during this stage of development is keeping pace with the growth rate of the larvae.

Treatment with exogenous T_4 significantly increased the T_4 concentrations in 5- and 20 ng/ml T_4-treated larvae compared with controls, with the highest levels observed during the natural T_4 peak. This suggests that exogenous T_4 was taken up by the developing larvae and that T_4 treatment amplified the natural T_4 surge. The changes in the T_4 concentrations in T_4-treated larvae during this period may reflect a change in hormone clearance rates, solubility, and permeability. Virtually nothing is known about hormone clearance rates in larval fishes and requires investigation.

Triiodothyronine concentration taken overall in the highest T_4-treated group was significantly ele-

vated above that of the control, but the difference was not significant at any specific time period. This increase in T_3 concentration in the 20 ng/ml T_4-treated larvae is most likely a result of increased substrate (T_4) and possibly 5′-D activity [18]. In contrast, Reddy [17] observed no significant elevation in the T_3 content via T_4 treatment.

Exogenous T_4 treatment significantly increased the anal depth to standard length ratio and the body area in the 20 ng/ml T_4-treated larvae compared with controls, whereas PTU inhibited these measurements. It is well established that exogenous thyroid hormone treatment affects growth and development in teleost fishes [1]. However, there were no significant effects of hormone treatment on growth (body weight and standard length). These results indicate that the T_4-treated larvae were growing more rapidly dorsal-ventrally compared with controls. One possible explanation for the anal depth to standard length and body area being the only significant morphometric changes is that these two measurements may be the most sensitive measures (i.e., additive effects of standard length to depth measurements—operculum depth, anal depth and caudal peduncle depth) to the effects of hormone treatment. Weber *et al.* (in preparation) observed an inhibitory effect of extremely high levels of T_3 (60- and 220-fold over that of controls at hatching) on larval body weight, in addition to other observed developmental effects that include effects on jaw development, increased pectoral fin growth and opercular deformities. Their study demonstrates that thyroid hormone treatment when given via injection (2.0- and 20 μg/g body weight) to the mother fish can affect the development of offspring in ways previously reported to be sensitive to thyroid hormones by immersion studies. The results of the present study provide further evidence for a role of thyroid hormones in morphogenesis of teleost fishes.

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